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14. ABSTRACT
Current therapies alter neuro-inflammatory/degenerative diseases (such as Multiple Sclerosis, MS) , however, little is known about their impact on neuroprotection and repair. Further, the development of effective therapies for these important components of MS has been hampered by the lack of sensitive clinical response criteria. We propose new methods aimed at associating changes in magnetic resonance (MR) detectable white/gray matter disease activity with pathological outcomes enhancing our understanding of degenerative processes resulting in tissue atrophy. We present novel protocols for existing MR scanning systems providing the ability to visualize structures that are impossible with current methods. Using techniques to concurrently stain and three-dimensionally analyze many cell types and structures related to auto-inflammatory pathology (glial cells, neurons, myelin, microvessels, infiltrates) and correlate these changes with existing and novel MR modalities will provide new ways of processing, acquiring, analyzing and understanding data from existing MRI hardware. Spinal cords from control mice and mice with induced autoimmune encephalomyelitis (EAE) were subjected to MR imaging using T1, T2, PD and DW protocols and newly developed MR imaging sequences. Novel imaging sequences were designed to resolve tissues with short T2 (invisible in conventional MRI) using new signal excitation methods. After MR acquisition, spinal cords were sectioned and immunofluorescently probed to concurrently label neurons, glial cells, microvessels, cellular infiltrates and myelin. 3D laser scanning microscopy and digital reconstruction were utilized to generate high resolution volumetric data. MR and multichannel microscopy data were co-registered and analyzed to identify novel cellular phenomena/structures related to autoimmune inflammatory diseases visible in the newly developed MR acquisition sequences that resolves tissue with a short T2.

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Introduction

Current consensus is that non-invasive techniques to track neuroprotection and repair in degenerative diseases of the central nervous system (such as Multiple Sclerosis, MS) are urgently needed. We propose to establish reliable and reproducible measures of neural tissue damage and neurodegeneration. These measures will allow us to more accurately non-invasively follow disease progression and evaluate therapeutic efficacy. Existing therapies alter MS disease characteristics, however, little is known about their impact on neuroprotection/repair (Zivadinov et al 2008) and the development of effective therapies for these important components of MS has been hampered by the lack of sensitive clinical response criteria. MRI shows great potential as a noninvasive imaging tool for MS. MRI signal intensity (SI) from different soft tissues varies with the density of water protons in the tissues. Currently, the primary method for diagnosing the extent to which multiple sclerosis is progressing in an afflicted individual is biomedical imaging and principally magnetic resonance imaging (MRI), MS lesions often show increased water content with the prolonged T1 and T2 relaxation. The effectiveness of this technique is limited by the ability of high resolution scanning devices to detect lesions in affected brain areas at an early stage of development. Such detection is critical since early diagnosis and treatment is essential for long term amelioration of disease progression. We are developing new confocal microscopic and micro-MRI techniques to image both nerve damage as well as chemical changes in surrounding tissues caused by this disease. Additional uses of 3-dimensional image display and feature extraction provides a new and unique system for analysis of affected brain regions and coupled with other imaging techniques and molecular measurements holds significant promise for developing new diagnostic tools for the clinician. Further, our new imaging approach will benefit the evaluation of the effectiveness of existing and new therapies. Because of the prevalence of this neurological disorder, a number of drug companies have developed new compounds for alleviating the symptoms and relapses of multiple sclerosis. This research is designed to generate and critically analyze innovative methods that will augment current patient assessment and drug efficacy. The completed objectives will create novel protocols for existing MR scanning systems providing researchers and clinicians the ability to visualize structures in ways that are otherwise unobtainable with current methods. Innovative 3D images of neurodegenerative activity will also be created and used to provide unique cellular correlates related to changes in newly developed MR signals. Using techniques to concurrently stain and three-dimensionally analyze many cell types/structures related to MS pathology (glial cells, neurons, myelin, microvessels, infiltrates), as well as, correlate these changes with existing and novel MR modalities will provide new ways of processing, acquiring, analyzing and understanding data from existing MRI hardware. The newly developed procedures will be immediately relevant and available to provide clinicians/researchers informative ways to visualize neuronal injury, known to precede brain atrophy. In addition, these strategies can be employed to monitor disease activity and therapeutic response. This approach will provide an enhanced understanding of how volumetric cellular changes are non-invasively detectable with enhanced MR imaging allowing us to evaluate many facets of MS including; modified microvascular permeability, neuronal degeneration/protection, glial health, myelin ensheathment, infiltrate activity, lesion type and identify/resolve biomarkers of activity and progression. Overall, our research will produce a potential breakthrough that will significantly impact the landscape of MR diagnostic imaging of multiple sclerosis, as well as many other neurodegenerative states.

Body

Experimental Procedures

Animals

Spinal cords frozen on dry ice from animals with experimentally induced autoimmune-encephalomyelitis and control animals were kindly provided by Dr. Wi Jung from the University of Calgary using established protocols described elsewhere (Dasilva and Yong, 2008). Control and day 15 EAE spinal cords from animals with disability scores of 4 were used in the current study.

Tissue Processing

Frozen spinal cords were fixed in 4% paraformaldehyde in PBS for 48 hours, after which they were placed in PBS and stored at 4°C until subjected to NMR. After NMR data acquisition spinal cord sections were embedded in 3% agar gel prior to slicing. 100 micron sections were acquired on a media cooled vibratome and stored in multiwell plates in PBS at 4°C until subjected to the tissue staining and analysis procedure.

NMR Imaging

Fixed spinal cord segments were placed within a small section (2 inch) of a 3mm NMR tube (Wilmad-Labglass, Vineland, NJ) which was then placed inside a 4mm NMR tube with a small piece of damp paper towel to prevent the sample drying during the acquisition process. Both tubes were next placed inside a 5mm NMR tube and subjected to the scanning procedure. NMR imaging data was acquired using a Varian 500Hz spectrometer equipped with a micro-imaging kit and using VarianJ imaging software. The best in-plane spatial resolution, up to 10 μ, has been achieved by using spin-echo based imaging pulse sequences: SEMS (spin-echo multi-slice) and its modification for diffusion-weighted imaging. Gradient-echo sequences provided comparable resolution but produced images with some distortion from non-perfect shims. For high-field magnet we used (12 T) shimming is a difficult problem, so we immersed our spinal cord samples into Fluorinert (Oakwood Products Inc.) for matching diamagnetic susceptibility. This reduced spectral width from 200 Hz to 40 Hz, greatly improving resolution and sensitivity. 0.5mm, or .25 mm adjacent slices spanning the entire region of the sample were acquired using the aformentioned protocols. NMR data was saved in the native .FDF format and visualized using NIH's ImageJ.

Immunostaining and Microscopy

Adjacent 100 micron sections from the samples were fluorescently labelled to identify numerous cell types and structures using validated antibodies/probes for comparison to the MR imaging data. Astrocytes, Oligodendrocytes, Neurons, Microglia, Macrophages, Microvessels, Myelin and Nuclei were stained in the sections to identify specific structures that were visible using the novel MR imaging procedures. Double and single antibody fluorescent staining procedures were utilized to label the tissue. Briefly, for double antibody labeling procedures, the samples were incubated in antibodies toward the cell types/structures of interest (at a concentration ranging from 1:50-1:2000 depending on the probes in PBS, 3% donkey serum and .5% Triton-X for 3 days in the fridge. After 3 X 15 minute washes in PBS, slices were incubated in the appropriately labeled secondary antibody and/or labeled primary antibodies in PBS for 48 hours. Sections were dried on a charged slides and mounted in Vectashield (with or without DAPI, where appropriate) mounting media and coverslips sealed with clear nail polish. Individual sections were scanned using a Fluoview F-1000 confocal scanning microscope equipped with 4 laser lines and wide specturm imaging capabilities. Image stacks were acquired

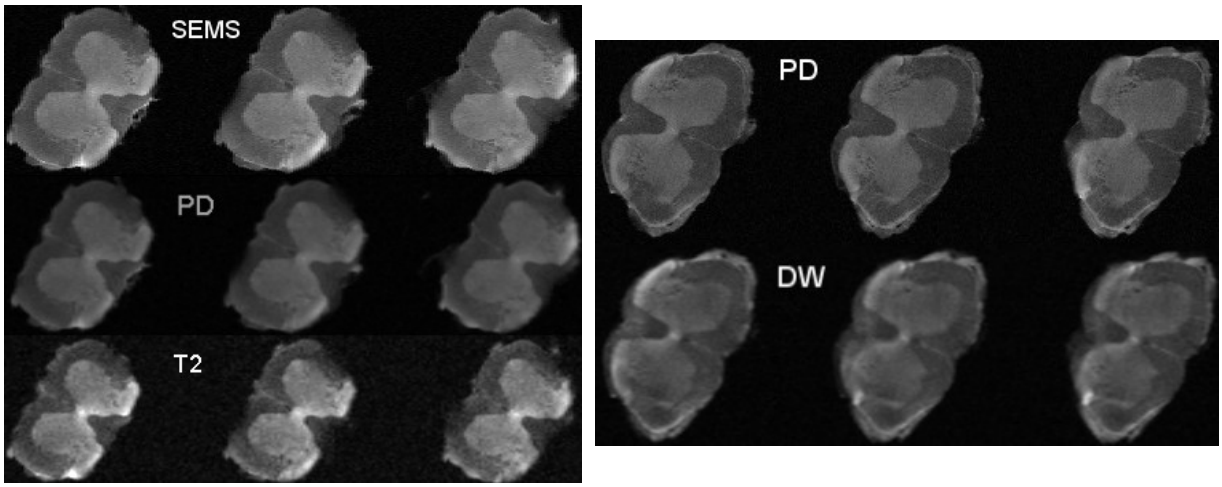
using 20X and 60X oil objectives. Tiled montaged image stacks were acquired using the 20X objective and the programmable stage in order to image entire spinal cord sections. Regions of specific interest as determined by tiled microscopy montages and NMR imaging data were scanned three dimensionally at higher resolution.

Analysis

MRI data was co-registered to the microscopy images using ImageJ's Unwarp plugin and visualized and analyzed with ImageJ and in house developed software. Based on the NMR imaging data matrix and the known size of slices confocal images corresponding microscopic images were co-regsitered and overlayed on top of the NMR data. Using the MR images as a guide, fluorescently stained structures were identified within regions of the novel MR signals.

Sample spinal cords were imaged using a number of standard MR protocols and custom designed sequences. Following MR imaging spinal cords were sliced with a media cooled vibratome, and processed for fluorescence microscopy. Samples were imaged for 2-3 experiments depending on the length of acquisition to prevent samples over heating and drying. Drying was typically not an issue with the samples for these periods and did not affect antibody binding or staining. Acquired sequences provided T1, T2, proton density, and diffusion-weighted contrasts. Figure 1 includes example MR images taken from a number of samples using the described standard protocols.

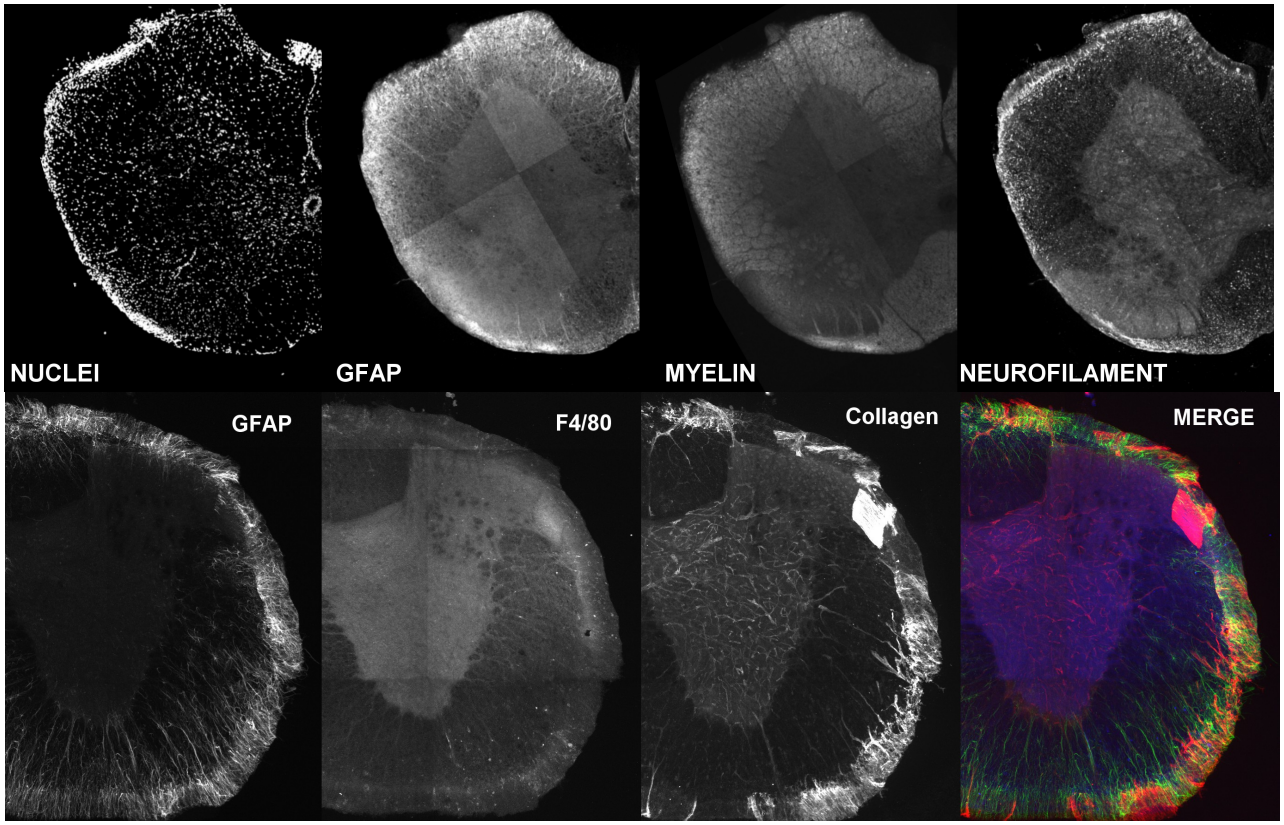
Figure 1. Spinal Cords were imaged using a variety of MRI modalities.



Sequential Images from spinal cord sections scanned with a variety of MR scanning methods including SEMS , Proton Density (PD) , T2 , Diffusion Weighted (DW) . Clearly from these images the protocols do not provide high contrast or resolution in all areas of the spinal cord. Our objectives for the new procedures is to enhance this contrast to better visualize structures of interest.

It was obvious from the samples that the major area lacking in contrast in each modality was gray areas within the tissue samples. Disease activity was typically visible as hyper intense regions within the white matter. All MR imaged spinal cords were subsequently mounted in 30% agar and sliced using a media cooled vibratome. Samples were fluorescently stained for numerous structures and cell types including neurons, activated microglia, endothelial cells, oligodedrocytes, astrocytes, microvessels, myelin and myelin components. Example micrographs of stained samples are outlined in Figure 2. Staining patterns consistent with known spinal cord structures most notably neurofilament, myelin, and microvessel segments staining (Figure 2) elucidated spinal anatomy.

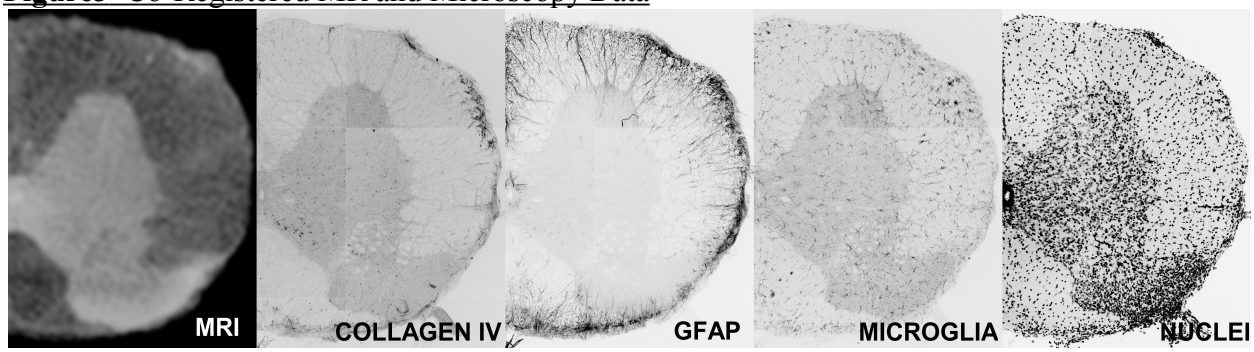
Figure 2. Fluorescently Stained and scanned spinal cord sections displayed the characteristic structure of the cross sected spinal cord



The above images are multichannel micrographs from sliced and stained spinal cords. Each image is created from tiled 3D data acquired from 9 optical fields using a 20X lens. Stained nuclei (DAPI) showed increased density within the cellbody rich grey matter areas as well as denser areas on the periphery of the spinal cord. Myelin filled the peripheral white matter regions of the spinal cord as expected. Neuronal cell bodies were visible in the central regions of the spinal cord as well as transected axons in the peripheral regions. Astrocytes typically formed a ring around the outside of the spinal cord and were in close association with the microvasculature as stained by collagen IV antibody.

In order to validate existing MR and fluorescence techniques spinal cord sections were stained, imaged and compared to MR data . Comparisons were made by initially by identifying the MR image from the stained physical slice and running ImageJ's registration plugin bUnwarpJ. The registration plugin warps the target image to fit the source image providing satisfactory image registration. Spinal cords were imaged using multiple modalities to provide a basis for classifying which structures were visible using MR techniques as compared to fluorescence data (Figure 3). We were able to achieve fine resolution using our imaging probe, better than typically published results.

Figure3 Co-Registered MR and Microscopy Data



After data co-registration congruent regions between the fluorescence and MR data were apparent. Myelin and neurofilament were clearly identifiable based on MR data as expected, further the microvasculature stained within the tissue could be seen as slightly hepertense regions within the MR modality. For the purpose of assessing neurological disease increasing contrast and resolution could provide major enhancements to our ability to non-invasively image on-going cellular phenomena.

MRI was also used to identify disease activity related to EAE induction in the experimental animals. Regions with disease activity were most easily seen as hypo or hyper intense regions in the MRI images of the spinal cord (Figure 6).

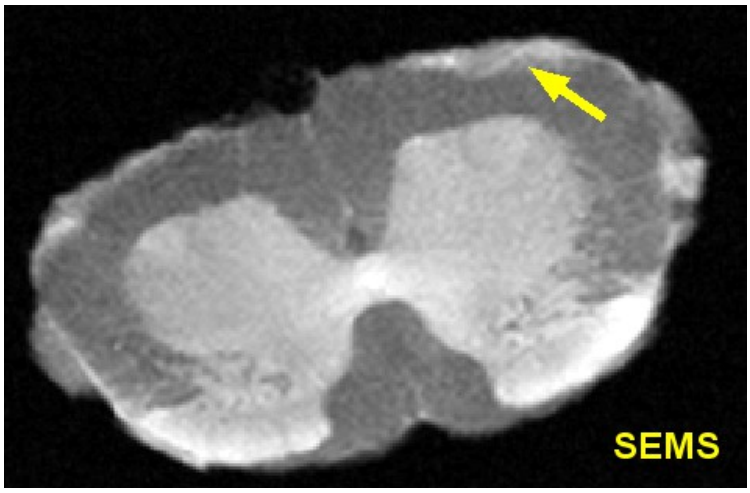
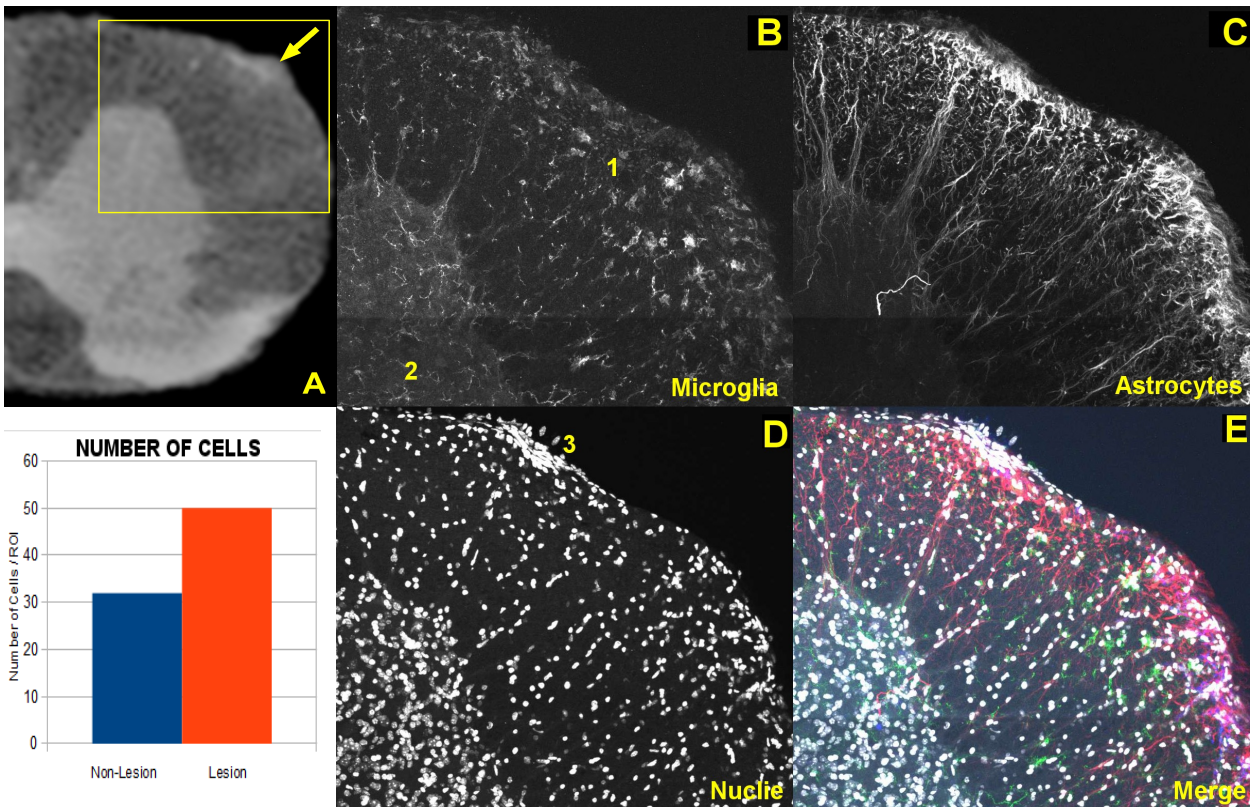


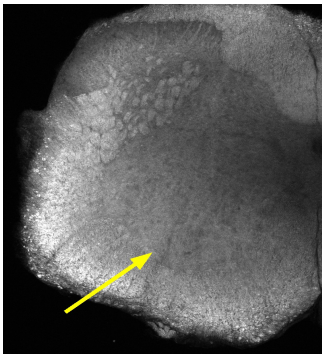
Figure 6. Visible Disease Activity in MR Images
MR images from the spinal cord of mice with induced EAE displayed visible lesions in areas of the white matter consistent with demylinating symptoms. Depending on the imaging modality, lesions were visible as hypo or hyper intense changes in MR signals. Data from MRI was used to identify disease activity and coincident slices . The above image is a SEMS image acquired from an EAE brain with a potential lesion, later confirmed using laser scanning microscopy.

Once disease activity was located to specific MR images the data was subsequently registered and compared to microscopy images from corresponding slices in order to both validate disease activity and identify visible structures. Disease activity was evident in microscopy images as a reduction in myelin staining, increased cellular infiltration, disruption of the blood brain and an increase in microglia with morphology consistent with the activated state. (Figure 7-9) and congruent with regions located in MR data.

Figure 7. Visible Disease Activity in Co-registered MR and Confocal Images



MRI data was used to identify demyelination and disease activity in the EAE spinal cord. Disease activity in a SEMS MR image was visible as hyperintense regions (A, arrow). Subsequent staining and comparison with co-registered microscopy displayed insights into cellular disease activity. (b)IBA-1 stained microglia display phenotypes consistent with the ameoboid activated state and could be clearly seen in the vicinity of the lesion (B,1)However, microglia in the gray matter had a ramified resting appearance (B,2) (C)Stained astrocytes were seen forming the blood brain barrier in close association with microvessels and microglia. (D) Lesional areas are also characterized by increased cellularity indicative of infiltration as indicated by nuclei staining (D,3) (E) Merged confocal channels clearly display increased cellularity and activated microglia (green). Importantly, only a portion of the disease activity is visible in the MR image; apparently the regions with a dramatic increase in cellularity. Areas with modest or little increase in cells but with relatively large increases in activated microglia are less apparent in the MR image. This affirms the need for contrast enhanced MR imaging to image and predict disease related phenomena.



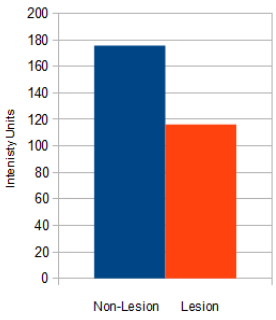
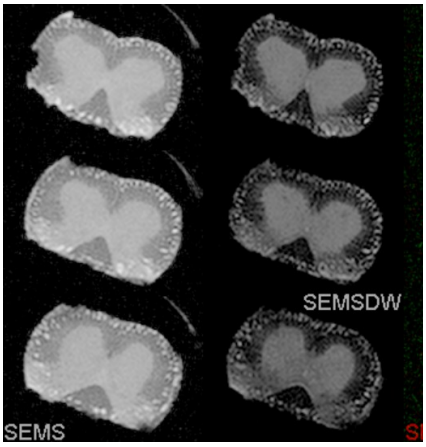


Figure 8. Subtle Myelin Changes Were not visible in MRI *The confocal micrograph to the left is an image from an EAE spinal cord stained for myelin. Small but significant changes in myelin content (arrow) in stained samples were not readily apparent in MR images when compared to microscopic images that also displayed cellular changes indicative of lesional activity. This affirms the need to enhance our ability to detect changes before current technology allows us to.*

Problems and Setbacks

During the course of the one year grant period we had a number of setbacks that we needed to overcome. Initially, we did not receive the funds to begin the project until well after the anticipated start date due to issues beyond our control (over 2 months). In addition, we had a number of problems associated with the Varian NMR imaging system. Specifically, we purchased totally new spectrometer, Agilent 500, using NSF grant CHE-1048645 (keeping only the old magnet, which is of high quality). Both the software and hardware are very different now. The vendor (Agilent) lost former Varian support personnel responsible for the added imaging capabilities of our original Varian instrument. As a result, we encountered a complete lack of technical support that required nearly 6 months of the combined efforts between Agilent engineers and Anatoly Khitrin, a participant in the current project, to resolve the issues. Other technical problems with the spectrometer were relatively minor and did not create significant delays. In addition to issues with the spectrometer, we encountered a number of sample related issues. Firstly, a number of spinal cords suffered from freezer related damage that displayed as punctate regions surrounding the outside of the spinal cord (Figure 9). These were visible in both experimental and control samples, and not indicative of any discernable or recognized disease activity.

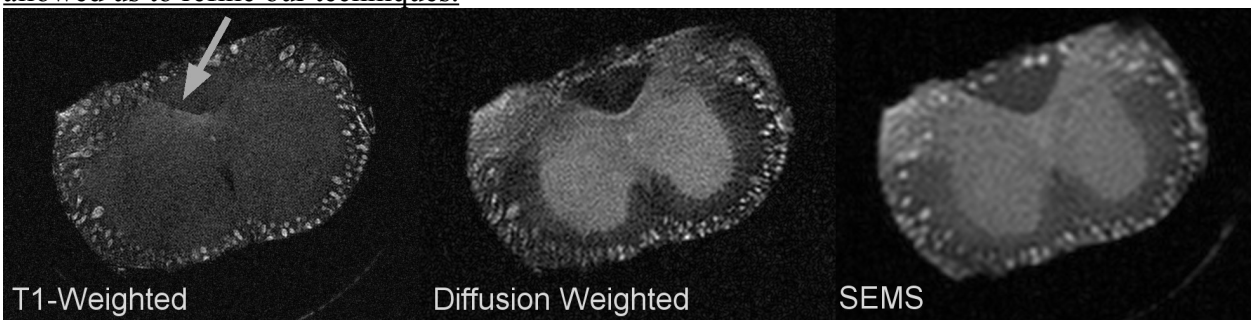
Figure 9. Damaged Samples Displayed a Punctate Appearance around the Spinal Cord



Approximately 20% of our sample population suffered from freezer related damage due to freezer freeze/thawing beyond our control. In the SEMS and Diffusion Weighted SEMS images to the right regions around the spinal cord could be seen as hyperintense damaged regions in the spinal cord. When imaged using confocal microscopy, these regions were seen to be physically damaged (both in control and EAE tissue) in regions. However, we were still able to use areas of the samples for this project.

This led to difficulty in assessing some regions of the spinal cord, however, we were able to use our new imaging techniques to visualize regions that were not damaged consistently between the control and experimental populations. In fact, our new methods allowed us to visualize structures in these damaged samples that were not visible using conventional techniques allowing us modify our protocols, and infer what new regions were becoming visible (Figure 10).

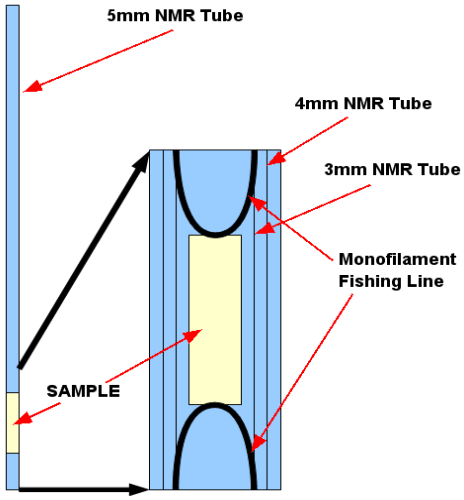
Figure 9. Damaged Samples Did Provide Guidance when Designing the New Protocols and allowed us to refine our techniques.



In the above image a damaged sample was used to refine our imaging technique and provide good resolution of structures using different modalities to highlight different regions. In this sample using the modified T1-Weighted protocol we were able to gain contrast in grey matter regions and locate an apparent grey matter area with apparent degenerative activity (arrow). Confocal microscopy confirmed that this region displayed modified neurofilament staining indicating disrupted neuronal function.

Secondly, in order to acquire quality MR image magnetic fields must be as homogenous as possible. In order for this to occur we needed to secure the spinal cords that were approximately 3mm in diameter within the 5mm tube without the sample moving. In order to accomplish this we needed to create a novel sample holding system. After much trial and error we settled on a design outlined in figure 10 to ensure the samples remained in the middle of the tube and did not move when being placed in and out of the imaging hardware.

Figure 10. We created a new way to secure samples within 5mm NMR tubes



In order to create homogenous magnetic fields and place the specimen at the correct position thus ensuring the best quality images possible we had to invent a new sample holding system within the 5mm NMR tubes. After numerous attempts we accomplished this by placing the spinal cord within a section of 3mm tube suspended at the top and bottom by a short section of looped monofilament fishing line (non magnetic). The tube was then placed within a section of 4mm tube and inserted into the 5mm tube. The sample was located at the optimal veritcal position and centered within the glassware.

Even after centralizing the samples in the NMR tube rig we still did not have adequate shims and as outlined above we immersed the sample in fluorinert that provided enhanced shims and contrast adding another discovered aspect to the project. The final related issue was the amount of time it took to acquire data from the samples with relatively poor shims. Typically, to acquire good resolution and a large amount of axial slices many signal averages (>100) needed to be acquired. Further, to prevent tissue overheating and hardware damage we were limited to approximately one pulse per second further slowing down acquistion. Typical acquisition times

were 16-30 hrs for our samples, however, this would not be the case for human systems due to relatively homogenous fields around the patient.

While we did encounter a number of issues, the most detrimental being the MR hardware downtime, they ended up providing areas for further innovation and development of our techniques and methodologies. The setbacks forced us to create novel solutions that will benefit future researchers in this field and actually increase the broader impacts of our research.

Key Research Accomplishments

- ➔ high resolution MR images of EAE disease activity using a number of standard protocols. Resolution significantly outperforming most published data attaining a resolution <15 microns
- ➔ co-registered 3D microscopic data associated visible disease activity
- ➔ information regarding what disease related activity is visible using current techniques, and more importantly which structures are not visible
- ➔ a novel system for securing the sample in NMR tubes better aligning the magnetic field
- ➔ new technique for shim enhancement using spinal cords immersed in liquids
- ➔ preliminary unique pulse sequences and MR methods designed to enhance contrast on existing hardware better imaging disease activity

Reportable Outcomes

- ➔ Society for Neuroscience 2012 Poster Presentation, New Orleans, Oct 2012;
- ➔ Early stage development of new contrast enhancing technique applicable to medical MRI systems.
- ➔ Novel technique for signal enhancement using samples imaged in small bore NMR hardware bathed in liquid contrast agent (fluorinert) designed to more closely mimic medical systems
- ➔ Custom designed NMR sample holding system to align magnetic fields and image samples.
- ➔ The study outcomes completed in the current grant will be used as pilot data for submission to one/all requests below new proposals;
    - ➔ National Science Foundation's Major Research Instrumentation Program. We intend to request a small animal MRI to continue our work into imaging neurodegenerative states in live animals and develop our techniques.
    - ➔ Multiple Sclerosis Society Pilot Research and Research grant programs
    - ➔ NIH Small Grant Program (R03)
- ➔ Peer reviewed research manuscript currently being authored, expected to be submitted in November 2012 "MR Imaging Techniques to Visualize Inflammatory and Degenerative Mechanisms."

Conclusion

We have created a new preliminary protocol that provides significant contrast enhancement in regions know to be affected by neurological disease states and otherwise invisible. This was accomplished by modifying acquisition parameters and pulse sequences that are currently available. Specifically, this was designed to enhance contrast around the edges of the clearly detectable MR lesions that were only visible when performing co-registered confocal imaging. In addition we provide preliminary data and imagery displaying our ability to enhance contrast in grey matter regions to image disease phenomena. These regions of the spinal cord may provide early feedback about neurological disease activity and areas that are either pre-lesional, or show modest disease activity. In particular the new technique should help image events early in neurological disease states including blood brain barrier leakage, early neuronal malfunction and the first steps of the auto-immune attack. Our next step is to begin imaging live animals more closely paralleling human MRI studies and provide a basis for human testing. The

benefit and potential impact of our findings are significant and far reaching; we envision an inexpensive software extension for currently installed MR imaging systems to provide enhanced visualization of disease states.

This research is designed to generate and critically analyze innovative methods that will augment current patient assessment and drug efficacy. The completed objectives will create novel protocols for existing MR scanning systems providing researchers and clinicians the ability to visualize structures in ways that are otherwise unobtainable with current methods. Innovative 3D images of neurodegenerative activity were also generated and analyzed and used to provide unique cellular correlates related to changes in newly developed and existing MR signals. Using techniques to concurrently stain and three-dimensionally analyze many cell types/structures related to MS pathology (glial cells, neurons, myelin, microvessels, infiltrates), as well as, correlate these changes with existing and novel MR modalities will further provide new ways of processing, acquiring, analyzing and understanding data from existing MRI hardware. The newly developed procedures will be relevant and available to provide clinicians/researchers informative ways to visualize neuronal injury, known to precede brain atrophy. In addition, these strategies can be employed to monitor disease activity and therapeutic response. This approach will provide an enhanced understanding of how volumetric cellular changes are non-invasively detectable with enhanced MR imaging allowing us to evaluate many facets of MS including; modified microvascular permeability, neuronal degeneration/protection, glial health, myelin ensheathment, infiltrate activity, lesion type and identify/resolve biomarkers of activity and progression. Overall, our research will produce a potential breakthrough that will significantly impact the landscape of MR diagnostic imaging of multiple sclerosis, as well as many other neurodegenerative states.

Multiple sclerosis (MS) is the second leading cause of neurological disease in young adults second only to trauma. It is a multi-focal and multi-phasic immune-mediated disorder characterized pathologically by inflammatory demyelination and neuronal injury. For many years the focus in MS research has been on inflammatory white matter pathology, since the disease was considered to be primarily an immune-mediated demyelinating disorder based on MRI as the main diagnostic and monitoring tool in MS patients. However, more recently histological approaches have demonstrated axonal damage and degeneration in post-mortem MS brains (Ferguson et al., 1997; Trapp et al., 1998) that appears to occur early in the disease supporting the involvement of neurodegenerative component in MS pathogenesis. Consistent with these findings, MRI studies have also revealed aggressive brain atrophy (Miller et al., 2002) and decreased N-acetylaspartate (NAA) that may indicate neuronal dysfunction (Bjartmar et al., 2000). Despite the significant correlation between axonal and neuronal degeneration with demyelinating lesions (Kornek et al., 2000; Peterson et al., 2001) there is also substantial data documenting the appearance of axonal damage in the absence of active demyelinating processes (Bitsch et al., 2000; DeLuca et al., 2006). Experimental Autoimmune Encephalitis (EAE) is a dominant animal model utilized in MS research. In EAE, axonal damage has been observed and patterns of this pathology and molecular abnormalities such as redistribution of ion channels on chronically demyelinated axons appear similar to those found in MS (Kornek et al., 2000, 2001) Further, it has been suggested that the mouse model of EAE exhibits greater CNS sensitivity with respect to axonal damage, thus, providing an advantageous model for investigating mechanisms regarding neurodegeneration in EAE (Gold et al., 2006). We intend to refine our developed procedures and protocols to better visualize objects currently invisible using conventional techniques with live animals and larger *post-mortem* samples Our goal is to produce compatible, innovative and non-invasive protocols to provide researchers and clinicians better ways to monitor and treat diseases.

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Appendices

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Novel mr imaging techniques to visualize inflammatory/degenerative mechanisms

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Current therapies alter neuro-inflammatory/degenerative diseases (such as Multiple Sclerosis, MS) , however, little is known about their impact on neuroprotection and repair. Further, the development of effective therapies for these important components of MS has been hampered by the lack of sensitive clinical response criteria. We propose new methods aimed at associating changes in magnetic resonance (MR) detectable white/gray matter disease activity with pathological outcomes enhancing our understanding of degenerative processes resulting in tissue atrophy. We present novel protocols for existing MR scanning systems providing the ability to visualize structures that are impossible with current methods. Using techniques to concurrently stain and three-dimensionally analyze many cell types and structures related to auto-inflammatory pathology (glial cells, neurons, myelin, microvessels, infiltrates) and correlate these changes with existing and novel MR modalities will provide new ways of processing, acquiring, analyzing and understanding data from existing MRI hardware. Spinal cords from control mice and mice with induced autoimmune encephalomyelitis (EAE) were subjected to MR imaging using T1, T2, PD and DW protocols and newly developed MR imaging sequences. Novel imaging sequences were designed to resolve tissues with short T2 (invisible in conventional MRI) using new signal excitation methods. After MR acquisition, spinal cords were sectioned and immunofluorescently probed to concurrently label neurons, glial cells, microvessels, cellular infiltrates and myelin. 3D laser scanning microscopy and digital reconstruction were utilized to generate high resolution volumetric data. MR and multichannel microscopy data were co-registered and analyzed to identify novel cellular phenomena/structures related to autoimmune inflammatory diseases visible in the newly developed MR acquisition sequences that resolves tissue with a short T2.